Efficacy of SEQ ID NO 7

Efficacy against infection:

Infection models are routinely used in antibiotic drug development as a rapid screening model for bacterial killing ability. While SEQ ID NO 7 and related peptides do not function by direct bacterial killing, efficacy in these models is an important demonstration of the ability of the peptide to induce a "protective" host response in a very acute and rapid infection. Specific discussion of the mechanisms by which SEQ ID NO 7 may elicit this protection is described in the attached recent publication in Nature Biotechnology (copy of paper attached).

SEQ ID NO. 7 has demonstrated efficacy in a number of infection models, with multiple pathogens, routes of administration and dosing regimes. Examples of this are included below.

- Efficacy in a mouse intra-peritoneal infection model (*S. aureus* strain 25923).

Figure 1. Female ICR mice were injected IP with 24 mg/kg SEQ ID NO 7 four hours after *S. aureus* 25923 (9.3 x10^9 cfu) had been injected in a 5% mucin suspension. The animals were sacrificed 24 hours after infection and the numbers of viable bacteria were assessed in peritoneal lavage fluid. Even though SEQ ID NO 7 has no direct bacterial activity, the numbers of recovered viable bacteria were reduced by approximately 3 orders of magnitude.

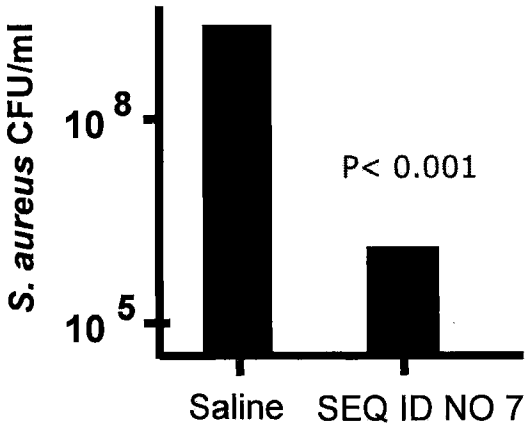


Figure 2. SEQ ID NO 7 (IMX) at 24 mg/kg was administered to female ICR mice at the indicated times before or after infection with S. aureus 25923 in 5% mucin. N=12 per group. Animals were sacrificed 24 hours after infection and bacterial counts were measured in peritoneal washings. Two separate experiments are shown. In the left panel, counts from individual mice are shown. Means are shown in the right panel.

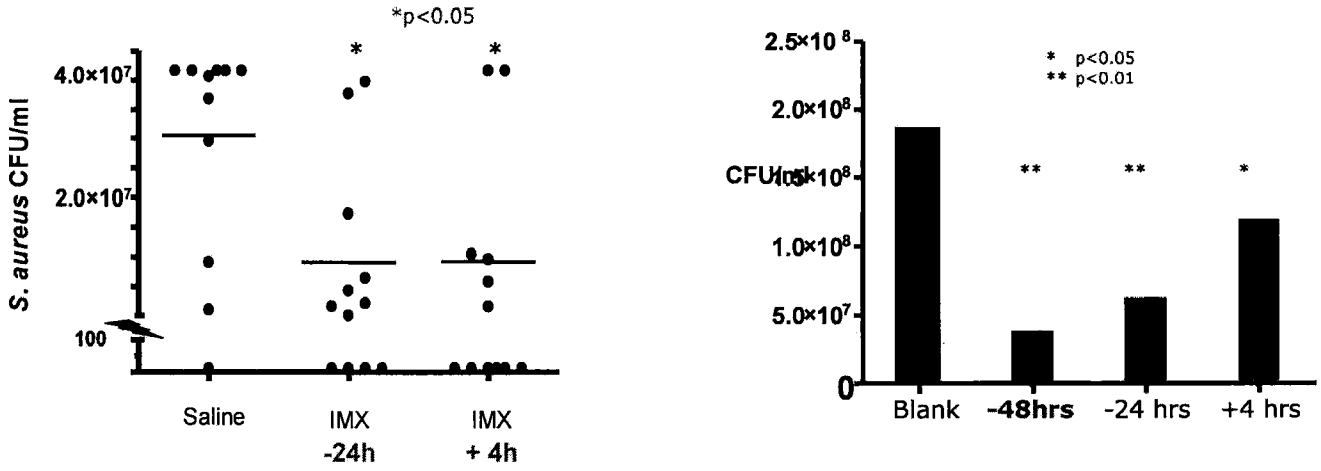
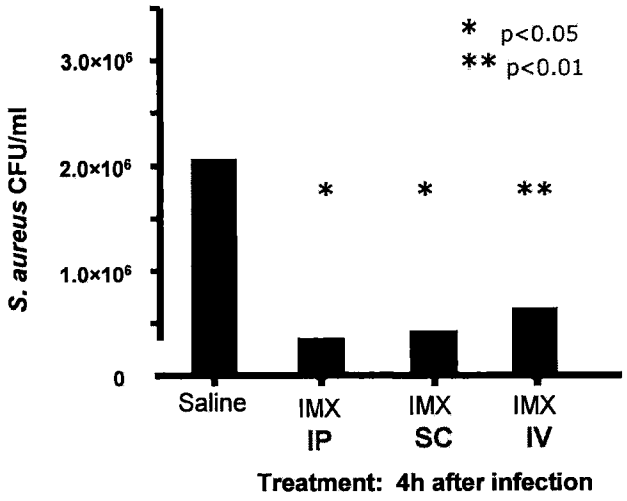


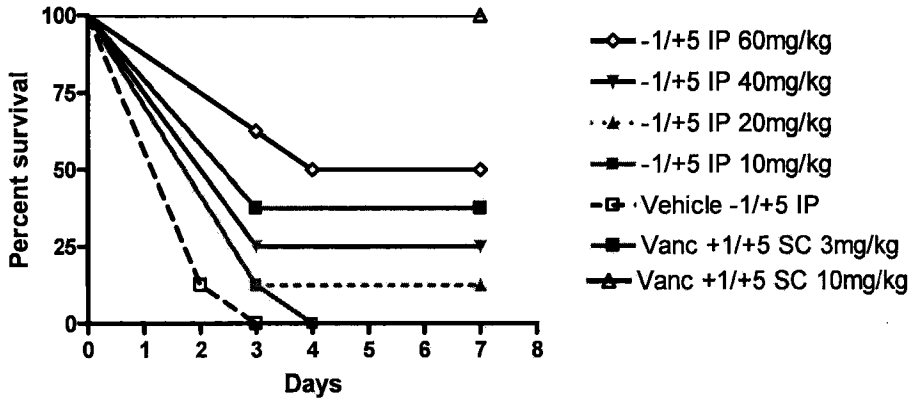
Figure 3. A comparison of Intraperitoneal, subcutaneous and intravenous administration of the peptide (SEQ ID NO 7; IMX) at 24 mg/kg, 4 hours after intraperitoneal infection with *S. aureus* in 5% mucin. All routes were effective, with differences between the routes being insignificant. N=8 female ICR mice / group.



• Efficacy against multiple pathogens

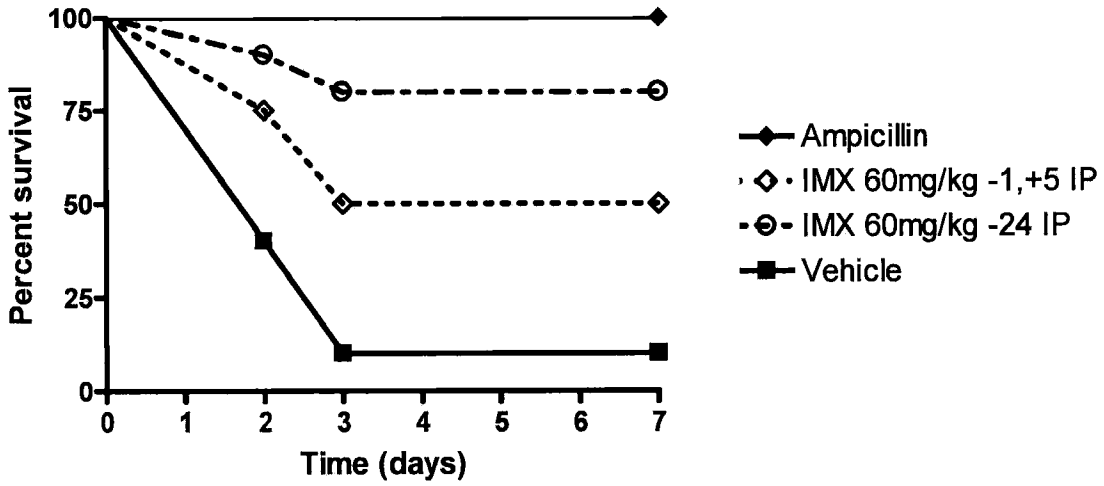
a. MRSA:

Figure 4. SEQ ID NO 7 was administered IP at various doses, one hour prior to, and 5 hours after, bacterial inoculation of $0.9x10^7$ S. aureus (ATCC 33591) with 5% mucin in male CD-1 (outbred) mice.



b. VRE:

Figure 5. CD–1 mice were infected with VRE by IP administration. SEQ ID NO 7 (IMX; open diamond) or vehicle (filled diamond) was administered IP 1h before and 5h after infection or 24h before infection (SEQ ID NO 7, open circle; vehicle, filled circle) with 1.8 x 10^8 CFU/mouse of VRE. Ampicillin was administered SC at 1 and 5 h post challenge. Survival was recorded over 7d. Survival curves were significantly different (P<0.01 by logrank test).



Efficacy against inflammation:

SEQ ID NO 7 has been observed to decrease inflammation in *in vivo* infection models (Figures 6, 7, 8) and acute inflammation models (Figure 9). These data have clearly indicated that in addition to aiding in the resolution of infection, SEQ ID NO 7 and related peptides are simultaneously able to modulate inflammation.

Figure 6. Mice injected IP with S. aureus in 5% mucin. 4 hours later, SEQ ID NO 7 (IMX) was injected. Mice were sacrified 18 hours later and the levels of TNFa in the peritoneal lavage fluid was assessed. Data from individual mice shown.

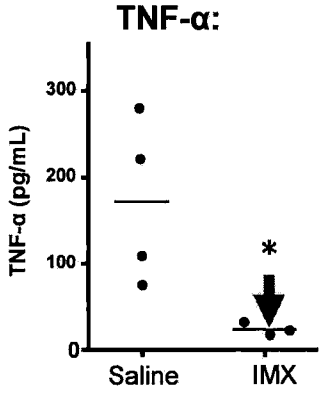


Figure 7. At time 0 all mouse groups were infected intra-nasally with 1.2 x 10^6 of bacterial CFUs. 24 mg/kg of SEQ ID NO 7 (IMX) was administered intra-peritoneally at the same time. In another group, AZM (azithromycin) was administered sub-cutaneously at 24 mg/kg 24 hours later. 48 hours after infection all mouse groups were sacrificed and BAL fluid was collected.
a) Bacterial counts in BAL fluid. b) TNF-α levels in BAL fluid. The geometric mean for each group is shown.

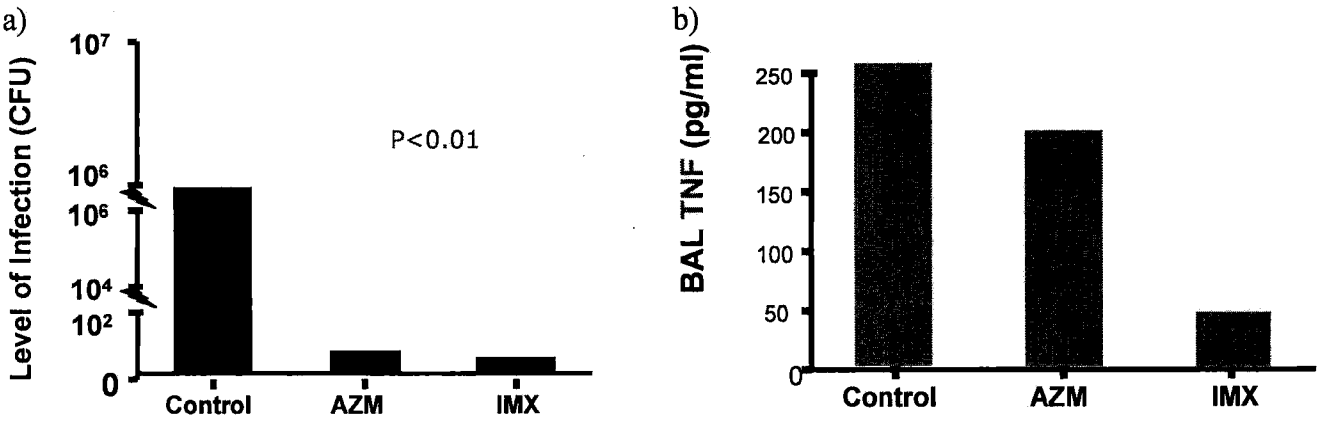


Figure 8: A bacterial inoculum of $1*10^8$ (A) or $5.7*10^8$ (B) S. aureus (ATCC 25923) with 5% mucin was administered in the peritoneal cavity (IP) to female ICR mice four hours (A, B) prior to or 24 hours after (B) intraperitoneal (IP) peptide administration (SEQ ID NO 7). Lavage samples were obtained 24 hours post-infection from surviving animals and analyzed for TNF-α (A; 05-130) and IL-6 (B; 05-048).

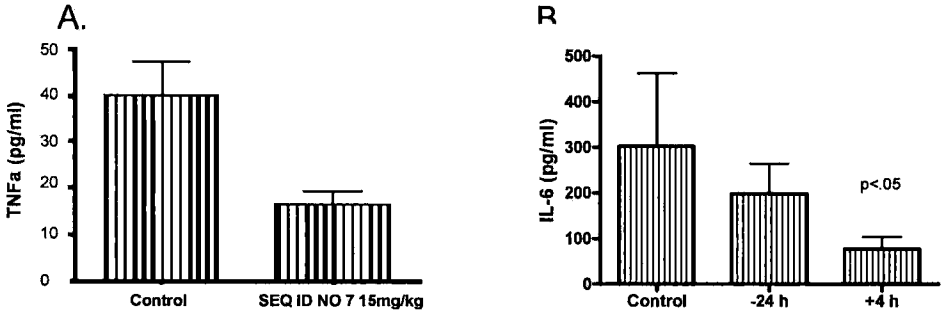
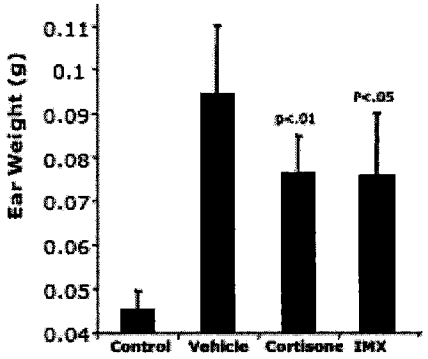


Figure 9: Ear inflammation was induced in CD-1 mice (5/group) with 5µg/20 µl PMA. Peptide (SEQ ID NO 7; IMX; 100 µg) or Cortisone (40 µg) was administered topically in 95% ethanol 30 min before and 30 min after PMA. Ear weight was measured 6 hrs after PMA.



Efficacy in Sepsis Models

As an extension of the anti-inflammatory activity elicited by SEQ ID NO 7 and related peptides, a reduction in sepsis is expected. This is demonstrated by the upregulation of CCL5 (RANTES; Figure 10), a positive prognosticator of sepsis outcome in a clinical setting[1], and by the activity of the related peptide, SEQ ID NO 6 in a sepsis model (Figure 11)

[1] Scand. J. Infec. Dis. 35(9): 535-44 (2003).

Figure 10: TranSignal human cytokine antibody array 3.0 (Panomics; MA6150) was used for the assessment of cytokine changes in the supernatants of peptide-treated cell cultures. THP-1 cells (ATCC, #TIB-202) were seeded into 6-well plates (2.5 x 106 cells/well), and treated with 60 ng/ml PMA for 18 hours. The cells were rested in DMEM/10% FBS for 24 hours, and then treated for 18 hours with SEQ ID NO 7 (IMX). The medium was removed and applied to a human cytokine array.

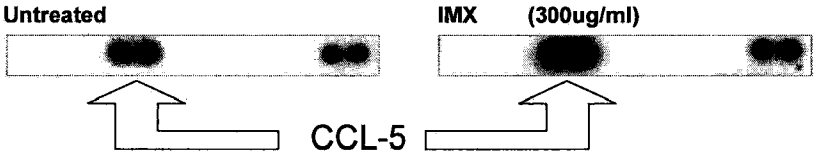
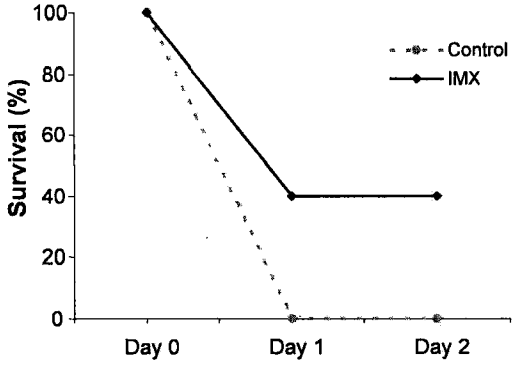


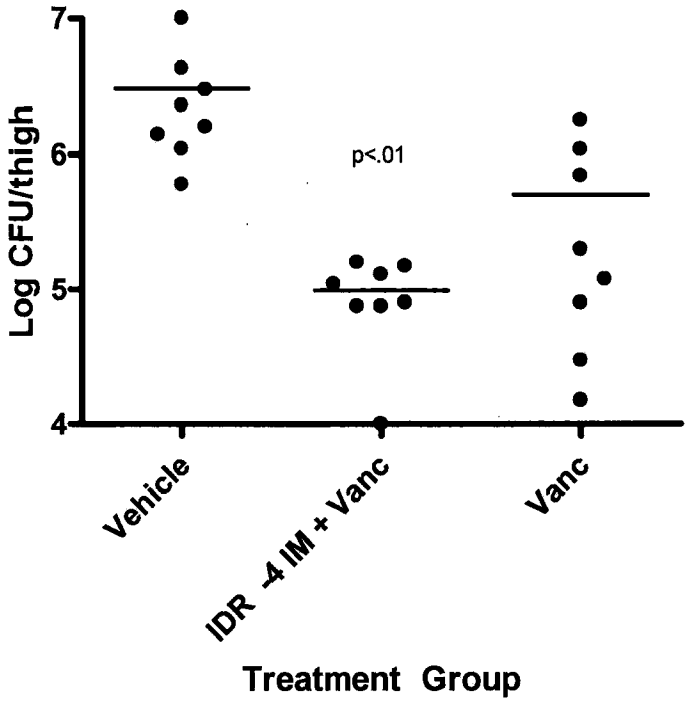
Figure 11: CD-1 (outbred) mice were injected IP with 200 µg LPS (endotoxin) and D-gal. SEQ ID NO 6 (IMX; 8.9 mg/kg) was injected at a separate IP site and survival was assessed daily; the other group received a saline (control) injection.



Complementary with Antibiotic Usage: Claim 93

When the host is unable to clear an infection *via* its immune system, antibiotics are administered to directly target the bacteria. In a complementary therapeutic approach, SEQ ID NO 7 and related peptides can augment the host's innate defences. This situation, where antibiotics alone are not sufficient to eradicate an infection, is represented in animals models using sub-optimal dosing of antibiotics (*e.g.*, Fig 1 in the application, Figure 12 below).

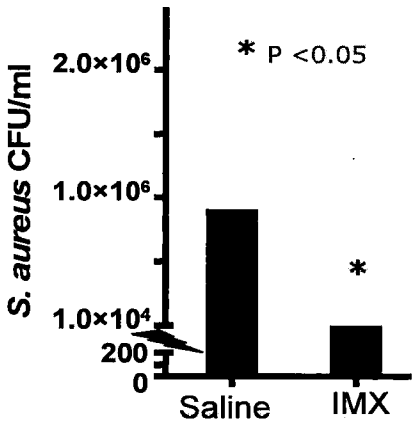
Figure 12: Female ICR mice (8/group) were injected with 1.8 x 10^6 CFU/mouse S. aureus (ATCC29213) mixed with cytodex beads. IDR (SEQ ID NO 7) was administered intra-muscularly 4 hours prior to infection. Vancomycin was administered sub-cutaneously 1, 6 and 24 hours after infection. Mice were euthanized 48 hours post-infection and bacterial counts in the thigh were determined.



Importantly, antibiotic administration is occasionally given prophylactically to patients at high risk for developing an infection (for example, patients which have undergone autologous stem cell transplantation are often given prophylatic antibiotics during their recovery period in addition to GM-CSF). Prophylactic administration of antibiotics, while necessary in some cases, does increase the likelihood of engendering antibiotic resistance, limiting the lifetime of current antibiotics and creating "superbugs". Thus, in these situations, it would also be advantageous to administer SEQ ID NO 7 and related peptides, thereby recruiting a more effective immune response. SEQ ID NO 7 is effective in immune-compromised situations, as shown in Fig. 13.

Figure 13.
A: Female RAG1 mice (lacking T and B cells) were injected IP with S. aureus in 5% mucin. 4 h later, 24 mg/kg SEQ ID NO 7 (IMX) was injected. Mice were sacrificed 24h later and the bacterial load in peritoneal lavage fluid was assessed.
B: Female CD1 mice were rendered neutropenic by treatment with 200mg/kg cyclophosphamide 4 and 1 day before intraperitoneal infection with S. aureus 25923 in 5% mucin. 24 mg/kg SEQ ID NO 7 (IDR) was administered IP 4 hours after infection and survival was assessed at 24hours. N=8

a)



b)